

Article

Associations of Obesity Measurements with Serum Metabolomic Profile: A Chinese Twin Study

Chunxiao Liao¹, Biqi Wang¹, Wenjing Gao^{1,*}, Weihua Cao¹, Jun Lv¹, Canqing Yu¹, Tao Huang¹, Dianjianyi Sun¹, Shengfeng Wang¹, Zengchang Pang², Liming Cong³, Hua Wang⁴, Xianping Wu⁵ and Liming Li^{1,*}

¹Department of Epidemiology and Biostatistics, School of Public Health, Peking University, Beijing, China, ²Qingdao Center for Diseases Control and Prevention, Qingdao, China, ³Zhejiang Center for Disease Control and Prevention, Hangzhou, China, ⁴Jiangsu Center for Disease Control and Prevention, Nanjing, China and ⁵Sichuan Center for Disease Control and Prevention, Chengdu, China

Abstract

The objective of this study was to investigate how different obesity measures link to circulating metabolites, and whether the connections are due to genetic or environmental factors. A cross-sectional analysis was performed on follow-up survey data at the Chinese National Twin Registry (CNTR), which was conducted in four areas of China (Shandong, Jiangsu, Zhejiang and Sichuan) in 2013. The survey collected detailed questionnaire information and conducted physical examinations, fasting blood sampling and untargeted metabolomic measurements among 439 adult twins. Linear regression models and bioinformatics analysis were used to examine the relation of obesity measures, including body mass index (BMI), waist circumference (WC) and waist-to-hip ratio (WHR) with serum metabolite levels and related pathways. A co-twin control study was additionally conducted among 15 obesity-discordant monozygotic (MZ) pairs (intrapair BMI difference >3 kg/m²) to examine any differences in metabolites controlling for genetic factors. Eleven metabolites were associated with BMI, WC and WHR after controlling for genetic and shared environmental factors. Pathway analysis identified pathways such as phenylalanine metabolism, purine metabolism, valine, leucine and isoleucine biosynthesis that were associated with obesity. A wide range of unfavorable alterations in the serum metabolome was associated with obesity. Obesity-discordant twin analysis suggests that these associations are independent of genetic liability.

Keywords: Obesity measures; serum metabolites; pathways; twin study

(Received 4 November 2020; accepted 17 December 2020)

Globally, the prevalence of obesity among adults has nearly quadrupled over the past four decades (NCD Risk Factor Collaboration, 2017). In 2017, 4.72 million deaths and 148 million disability-adjusted life years (DALYs) were attributable to high body mass index (BMI) (GBD 2017 Risk Factor Collaborators, 2018). Genetic components and unhealthy lifestyles undoubtedly play a key role in the development of obesity; however, the pathogenic mechanism has not yet been fully explained (Zhang et al., 2012). In recent years, with the continuous emergence of new technologies, the rapid development of omics research may help to obtain a detailed molecular view of the pathology of complex diseases.

Metabolomics is a powerful tool to investigate the relationships between serum metabolites and disease states. Recent advances have allowed for high-throughput targeted or untargeted exploration of the metabolite changes in body fluids and tissues that may reflect physiological dysfunction and mirror earlier stages of metabolism disorders. In recent years, metabolomics is increasingly used to

explore the pathology of metabolic diseases like obesity (Kraus et al., 2016; Moore et al., 2014; Pietiläinen et al., 2007; Rauschert et al., 2014). Aromatic amino acids (AAA) (Ho et al., 2016; Moore et al., 2014; Short et al., 2019), branched-chain amino acids (BCAA; Ho et al., 2016; Newgard et al., 2009; Short et al., 2019), non-esterified fatty acids (Newgard et al., 2009; Zeng et al., 2010), acylcarnitines (Mihalik et al., 2012) and phospholipids (Pietiläinen et al., 2007) were reported to be associated with obesity. These metabolites might indicate important biological processes or represent intermediate phenotypes linking genetic and environmental factors of obesity and its related diseases.

Twin studies are unique for identifying genetic and environmental determinants of human phenotypes or traits due to identical (monozygotic; MZ) or partly shared (dizygotic; DZ) genome backgrounds. Previous twin studies showed genetic factors explain 54–81% of the interindividual variation in BMI (Nan et al., 2012; Zhou et al., 2015) while heritability for most serum metabolites ranged from 21% to 79% (Kettunen et al., 2012), demonstrating a genetic basis for individual differences in serum metabolite concentrations.

To the best of our knowledge, most of the evidence of metabolomic study on obesity has been from the European populations. Thus, using Chinese adult MZ twins, we aimed to identify obesity-related serum metabolites and potential metabolic pathways.

Author for correspondence: Wenjing Gao, Email: wenjinggao@bjmu.edu.cn; Liming Li, Email: lmlee@vip.163.com

*Liming Li and Wenjing Gao are the co-corresponding authors.

Cite this article: Liao C, Wang B, Gao W, Cao W, Lv J, Yu C, Huang T, Sun D, Wang S, Pang Z, Cong L, Wang H, Wu X, and Li L. (2021) Associations of Obesity Measurements with Serum Metabolomic Profile: A Chinese Twin Study. *Twin Research and Human Genetics* 24: 14–21, <https://doi.org/10.1017/thg.2021.3>

Methods

Study Population and Design

The Chinese National Twin Registry (CNTR) is one of the largest population-based twin registries in China. In 2013, a questionnaire survey, physical examinations and fasting blood sampling were conducted among 1147 adult twin pairs across 4 areas of China (Shandong, Jiangsu, Zhejiang and Sichuan). Furthermore, DNA was collected and whole-genome genotyping was conducted on 469 twins. The zygosity of these twins was determined by DNA analysis. The study has been described elsewhere in detail (Li et al., 2013). All participants provided written informed consent. The Biomedical Ethics Committee at Peking University (Beijing, China) approved the study.

Metabolomics detection met the following inclusion criteria in this study: aged more than 18 years; reared-together twins; blood donors without histories of cardiovascular disease and cancer; DNA determined zygosity information. Thus, we finally included 439 twins containing 215 twin pairs for this analysis.

Obesity Measurements

Obesity measures collected in this study were BMI, waist circumference (WC), waist-to-hip ratio (WHR) and percent body fat (%BF). Standing height and weight of twins was measured using a stadiometer (0.1 cm) and a digital balance (TANITA, Tokyo, Japan, 0.1 kg), respectively. BMI was calculated as weight in kilograms divided by height in meters squared. WC and hip circumference were measured twice using a nonstretchable tape. WC was measured at the umbilicus, while hip circumference was at the widest part of the buttocks and the mean values were used in the analyses. WHR was the ratio of WC and hip circumference. The %BF was measured by bioelectrical impedance (Body Composition Analyzer/Scale, TANITA).

Biochemical Measurements

Venous blood samples were drawn from the study subjects after a 12-hour fast. Serum total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), glucose (Glu) and serum insulin were measured in this sample, described in detail elsewhere (Liao et al., 2015). Insulin resistance was estimated according to homeostasis model assessment (HOMA-IR; $\text{HOMA-IR} = [\text{fasting glucose (mmol/l)} \times \text{insulin (U/ml)}] / 22.5$). To minimize the effects of assay variability, samples from each twin pair were analyzed using the same assay.

Untargeted Metabolomic Measurements

Sample preparation and analysis. High-resolution metabolomics profiling of MZ and DZ twins was completed using liquid chromatography with high-resolution mass spectrometry. All serum samples were analyzed with a 2777C Ultra (High)-Performance Liquid Chromatography (UPLC) system (Waters, Massachusetts, USA) connected to an SYNAPT G2 XS QTOF mass spectrometer (Waters, Massachusetts, USA) with electrospray ionization (ESI).

A quality control (QC) sample was made by mixing and blending equal volumes (20 μL) from each serum sample. QC was used to represent all the analytes encountered during analysis. For serum samples, 100 μL of the sample was mixed with 200 μL of methanol to precipitate protein. The mixture was then centrifuged

at 14,000 g for 10 min at 4°C. The supernatant was transferred into a 1.5 ml polypropylene tube for the following metabolic profiling experiment. In order to ensure system equilibrium, 10 pooled QC samples were injected at the beginning of the experiment. The QC samples were injected between every 10 samples to monitor system stability during the whole experiment. Metabolite features were detected with an SYNAPT-G2XS QTOF mass spectrometer in both positive and negative ion modes.

Data Processing and Identification of Metabolites

The raw tandem mass spectrometry datasets were processed using commercial software Progenesis QI 2.0 (Nonlinear Dynamics, Newcastle, UK), consisting of raw data import, selection of possible adducts, peak alignment, peak detection, deconvolution, dataset filtering and noise reduction. Intensities of each peak were recorded and a three-dimensional matrix containing arbitrarily assigned peak indices (retention time and m/z pairs), sample names (observations) and ion intensity information (variables) was generated. In order to obtain consistent results, the obtained matrix was further reduced by removing peaks with more than 80% missing values (ion intensity = 0). PCA was performed for outlier detection and batch effects evaluation using the preprocessed dataset. As a quality assurance strategy in metabolic profiling, all retained peaks were normalized to the QC sample using Robust Loess Signal Correction (R-LSC) based on the periodic analysis of a standard biological QC sample together with the real plasma samples. The relative standard deviations of the metabolic features were calculated across all QC samples, and those >30% were then removed. The untargeted metabolic analysis yielded 5580 features in the positive ion mode and 5634 features in the negative ion mode. Normalization by median was used as sample normalization and log transformation, and scaling by standard deviation was used as data normalization. Metabolites were confirmed by comparison of accurate mass m/z , retention time and ion intensity to online human metabolome databases (www.hmdb.ca, level 2 confirmation) or authentic reference standards (level 1 confirmation) and only these confirmed metabolites (76 features in the positive ion mode and 73 features in the negative ion mode) were used in the following analysis. Positive ESI and negative ESI data were analyzed and summarized separately.

Assessment of Covariates

We obtained covariates from questionnaire, including socio-demographic characteristics (age, sex, region and educational level) and lifestyle behaviors (tobacco smoking, alcohol drinking, diet and physical activity). Region was assessed by place of residence (Shandong, Zhejiang, Jiangsu, Sichuan provinces). Educational level was defined as illiterate, primary school graduated, secondary school graduated, high school graduated and above. Tobacco smoking was coded into three categories (never, former, current) according to participants' responses to 'Do you smoke?'. Alcohol drinking status was similarly defined depending on their responses to 'Do you drink alcohol?'. Physical activity was measured as a quintile of weekly metabolic equivalent of tasks measured by a Chinese version of the International Physical Activity Questionnaire (Macfarlane et al., 2011). Diet was measured by three variables: the frequency of having red meat in the past year, quintile of weekly fresh fruit (grams) consumption and quintile of weekly fresh vegetable (grams) consumption.

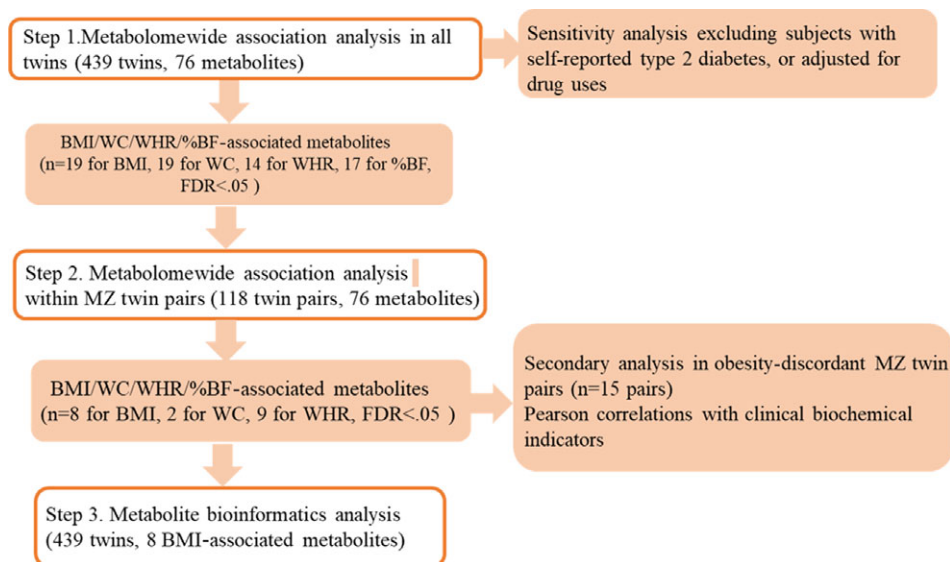


Fig. 1. Analyses framework of metabolomewide association analysis of obesity measures. Note: MZ = monozygotic; BMI = body mass index; WC = waist circumference; WHR = waist-to-hip ratio; %BF = percent body fat.

Statistical Analysis

Metabolomewide association study of obesity measures. Linear mixed models were implemented to explore the associations between metabolites and multiple obesity measures. We modeled each metabolite as a function of the log-transformed BMI, WC, WHR or %BF using linear mixed models adjusted for age, sex, zygosity, study sites, smoking, drinking, diet, physical activity, education and test batch as fixed effects, and potential correlation within twin pairs was accounted for by including a random intercept (Carlin *et al.*, 2005).

A sensitivity analysis was conducted by comparing the results with subjects without self-reported type 2 diabetes, or additionally adjusted for hypoglycemic/hypolipidemic drug use in recent months.

Second, to investigate whether these associations were confounded by genetic factors, we applied linear fixed models within MZ twin pairs adjusting for study sites, smoking, drinking, diet, physical activity, education and test batch. The within-twin-pair approach automatically takes into account shared genetic and environmental influences.

To better take the advantages of twins, we used an empirical Bayes-paired moderated *t* test to detect differential metabolites between obesity-discordant MZ twin pairs that were found in the above MZ twin-pair analysis. Discordant for obesity was defined as a BMI of 27 kg/m² or higher in one twin while being less than 24 kg/m² in their co-twin (Δ BMI > 3 kg/m²).

All analyses were implemented in R 3.1.2 and we set the threshold for statistical significance at a false discovery rate (FDR) *q* value of 0.05 or less, accounting for all metabolites.

Pearson correlations between metabolites with clinical biochemical indications. The correlations between metabolites and clinical variables were calculated using Pearson correlations both individually and by twin-pair among MZ twins. The logarithmic transformation was carried out to better conform to the normal distribution. In within-pair analysis, biochemical variables and metabolomics data were twin-normalized by taking the within-pair difference of the log₂-transformed concentration values for each variable. The reported *p* value is the probability of getting a correlation as large as the observed value by random chance, when

the true correlation is zero. All analyses were implemented in R 3.1.2 and the levels of significance are marked as *p* < .05.

Bioinformatics analysis. MetaboAnalyst 4.0 (<http://www.metaboanalyst.ca/MetaboAnalyst/>) was used to perform informatics analysis. MetaboAnalyst combines the results from powerful pathway enrichment analysis with the pathway topology analysis to discover the relevant pathways. The module of pathway analysis was based on the KEGG database. The *p* value and impact value threshold, as calculated via the analysis of pathway topology, were set to 0.05 and 0.1. Metabolite set enrichment analysis (MSEA) was performed based on Small Molecule Pathway Database (SMPDB, <http://smpdb.ca/>) using over-representation analysis (ORA) algorithms to identify biologically meaningful patterns significantly enriched in quantitative metabolomics.

All analyses described above were summarized in Figure 1.

Results

The study participants collected 439 twins containing 215 twin pairs (118 MZ twin pairs), with an average age of 45.1 years (range 18.0–80.9 years, 66.5% were males and 54.9% MZ twins; Table 1).

Metabolomewide Association Study of Obesity Measurements

We first regressed 76 metabolites identified in negative ion mode against obesity measures (BMI, WC, WHR, %BF) in all twins. We defined 69 associations that remained significant after FDR adjustment (Supplementary Table 1). The 69 associations were formed between 23 metabolites and obesity measures. Sensitivity analysis further adjusted for hypoglycemic or hypolipidemic drugs did not substantially change our results. When participants with type 2 diabetes were excluded, the associations of obesity measures with the hexoses and other metabolites remained significant (Supplementary Table 2a and b).

To assess whether any of the significant associations between the metabolites and obesity measures were confounded by genetic or shared environmental factors, we performed within-twin-pair comparisons using all 118 MZ twin pairs; 19 of the 69 associations remained significant after FDR adjustment, which was formed between 11 metabolites and the obesity measures (Table 2).

Table 1. Characteristics of the 439 twins

Characteristics	Mean \pm SD, or n (%)
Age (years)	45.1 \pm 13.2
Men	292 (66.5)
Monozygotic twins	241 (54.9)
Body mass index (kg/m ²)	25.0 \pm 3.8
Waist circumference (cm)	87.0 \pm 10.1
Waist-to-hip ratio	0.90 \pm 0.06
Percent body fat	26.7 \pm 7.6
Current smokers	141 (32.1)
Current regular drinkers	149 (33.9)
Vegetable consumption (grams/week)	1580.0 \pm 963.4
Fruit consumption (grams/week)	1096.2 \pm 1121.5
Physical activities (Metabolic Equivalent of Task, MET/week)	5808.6 \pm 5236.0
Red meat consumption	
Rarely/monthly consumed	70 (15.9)
One–three times per week	110 (25.1)
Four–six times per week	48 (10.9)
Once per day	131 (29.8)
Two or more times per day	77 (17.5)
Education	
Illiterate	51 (11.6)
Primary school graduated	73 (16.6)
Middle school graduated	213 (48.5)
High school graduated	66 (15.0)
College/University or more	36 (8.2)
Study sites	
Qingdao	129 (29.4)
Zhejiang	120 (27.3)
Jiangsu	143 (32.6)
Sichuan	47 (10.7)

Note: Current smokers: someone who smoked one or more cigarettes per day in the past half year; current regular drinkers: someone who drank more than 20 grams of ethanol per week in the past half year.

Eight significant associations (L-phenylalanine, L-isoleucine, L-tyrosine, uric acid, N-methyl-D-aspartic [NMDA] acid, C6H12O6, D-tryptophan and L-glutamine) remained in analysis with BMI, two significant associations (L-isoleucine and L-tyrosine) remained in analysis with WC and nine significant associations (L-tyrosine, NMDA acid, L-isoleucine, uric acid, L-phenylalanine, C6H12O6, PE(20:2(11Z,14Z)/0:0), PE(20:1(11Z)/0:0) and suberic acid) remained in analysis with WHR. These associations are independent of confounding by factors shared by the co-twins, and are rather due to unique environmental factors experienced by the individual twins.

According to our definition of ‘discordant obesity’, only 15 pairs of MZ twins were found with this condition. The distribution of obesity measures and clinical blood biochemical indicators are presented in Supplementary Table 3. Besides obesity measures, the serum level of insulin was different between the two groups. Most

of the metabolites were differential between obesity-discordant MZ twin pairs (Table 3).

In terms of the analysis in positive ion mode, we defined seven associations that remained significant after FDR adjustment (Supplementary Table 4). These associations were formed between three metabolites and obesity measures. Excluding participants with type 2 diabetes or further adjusting for hypoglycemic or hypolipidemic drugs did not substantially change our results (Supplementary Table 5a and b). Within-twin-pair linear regression and matched case control of obesity-discordant MZ twins did not identify any significant metabolites.

Pearson Correlation between Metabolites with Clinical Biochemical Indications

The analyses showed positive correlations of L-phenylalanine, L-isoleucine and L-tyrosine with serum insulin, HbA1c, HOMA, TC, TG and LDL-C; whereas, L-glutamine showed negative correlations with the abovementioned biomarkers (Table 4). The correlation between L-isoleucine and TC was significant in MZ twin-pair analysis as well as C6H12O6 with HDL-C, LDL-C and Glu. Most of the correlations were weakened in the twin-pair analysis.

Metabolic Pathway Analysis and Biological Significance Interpretation

The result of pathway analysis is shown in Figure 2A and Supplementary Table 6, and 10 metabolic pathways were identified, which relates to the proportion of phenylalanine metabolism that is significantly perturbed, followed by tyrosine metabolism, valine, leucine and isoleucine biosynthesis and purine metabolism. Furthermore, in order to expand the understanding of metabolic pathway related to BMI, the module of enrichment analysis was used, which found six additional pathways, including catecholamine biosynthesis, thyroid hormone synthesis, nucleotide sugars metabolism, sphingolipid metabolism, galactose metabolism and lactose degradation significantly related to BMI (Figure 2B and Supplementary Table 7).

Discussion

The present metabolomewide association study based on serum blood samples identified several obesity-related metabolites using untargeted metabolomic measurements in a Chinese twin sample. Eleven metabolites were associated with BMI, WC and WHR after controlling for genetic and shared environmental factors. Bioinformatics analysis identified pathways such as phenylalanine metabolism, purine metabolism, valine, leucine and isoleucine biosynthesis were associated with obesity. Besides, isoleucine showed a significant positive correlation with TC in MZ twin-pair analysis.

We first identified metabolites that were significantly associated with different obesity measures in individual twins. Many of these obesity-associated metabolites were organic acids and derivatives followed by lipids/lipid-like molecules. BMI was associated with more metabolites than any of the other studied obesity measures (WC, WHR, %BF) in this study. We found that 80% of metabolites associated with WC was also associated with BMI, and nearly all the metabolites associating with %BF was also associated with BMI. Besides, all the metabolites associated with WHR were associated with both WC and BMI. These overlaps in the metabolites reflect known high correlations between the different obesity measures (Menke et al., 2007).

Table 2. Significant metabolites with obesity measures in monozygotic within-twin-pair analysis

RT_M/Z	Metabolite_ID	Metabolite_name	Metabolite category	FDR	<i>p</i> value	logFC
BMI						
3.26_164.0712m/z	HMDB00159	L-phenylalanine	Organic acids and derivatives	1.04E-04	3.38E-06	3.481
1.51_130.0871m/z	HMDB00172	L-isoleucine	Amino acids and derivatives	1.04E-04	3.78E-06	3.498
1.23_180.0660m/z	HMDB00158	L-tyrosine	Amino acids and derivatives	1.04E-04	4.10E-06	3.522
0.93_167.0205m/z	HMDB00289	Uric acid	Organoheterocyclic compounds	4.49E-04	2.95E-05	2.877
0.58_146.0456m/z	HMDB02393	N-methyl-D-aspartic acid	Amino acids and derivatives	6.24E-03	5.29E-04	2.110
0.59_215.0322m/z	HMDB00143	Galactose/mannose/glucose	Hexoses	6.24E-03	5.75E-04	2.150
3.75_203.0819m/z	HMDB13609	D-tryptophan	Organoheterocyclic compounds	2.84E-02	2.99E-03	2.195
0.56_145.0615m/z	HMDB00641	L-glutamine	Organic acids and derivatives	2.99E-02	3.54E-03	-2.311
WC						
1.23_180.0660m/z	HMDB00158	L-tyrosine	Amino acids and derivatives	1.74E-02	4.26E-04	2.943
1.51_130.0871m/z	HMDB00172	L-isoleucine	Amino acids and derivatives	1.74E-02	4.57E-04	2.898
WHR						
1.23_180.0660m/z	HMDB00158	L-tyrosine	Amino acids and derivatives	4.36E-04	5.74E-06	3.969
0.58_146.0456m/z	HMDB02393	N-methyl-D-aspartic acid	Amino acids and derivatives	1.53E-03	4.02E-05	2.837
1.51_130.0871m/z	HMDB00172	L-isoleucine	Amino acids and derivatives	1.70E-03	6.70E-05	3.576
0.93_167.0205m/z	HMDB00289	Uric acid	Organoheterocyclic compounds	2.90E-03	1.91E-04	2.933
3.26_164.0712m/z	HMDB00159	L-phenylalanine	Organic acids and derivatives	7.90E-03	6.24E-04	2.676
0.59_215.0322m/z	HMDB00143	Galactose/mannose/glucose	Hexoses	2.21E-02	2.39E-03	2.411
8.63_504.3094m/z	LMGP02050021	PE(20:2(11Z,14Z)/0:0)	Lipids and lipid-like molecules	2.21E-02	2.54E-03	-2.683
8.94_506.3251m/z	LMGP02050020	PE(20:1(11Z)/0:0)	Lipids and lipid-like molecules	2.21E-02	2.62E-03	-2.515
4.89_173.0814m/z	HMDB00893	Suberic acid	Lipids and lipid-like molecules	4.39E-02	5.78E-03	1.685

Note: RT_M/Z = retention time(min)_mass-to-charge ratio; BMI = body mass index; WC = waist circumference; WHR = waist to hip ratio. logFC is the log fold change of metabolite values that associates with the ratio of the higher phenotype value over the lower phenotype values. The regression analysis was using linear fixed model adjusting for smoking, drinking, diet, physical activity, education and test batch for each phenotype.

Table 3. Differential metabolites in obesity-discordant monozygotic twin-pair analysis

RT_M/Z	Metabolite_ID	Metabolite_name	Metabolite category	FDR	<i>p</i> value	logFC
0.93_167.0205m/z	HMDB00289	Uric acid	Organoheterocyclic compounds	5.11E-03	6.39E-04	1.053
1.23_180.0660m/z	HMDB00158	L-Tyrosine	Amino acids and derivatives	1.03E-02	2.58E-03	1.076
0.58_146.0456m/z	HMDB02393	N-Methyl-D-aspartic acid	Amino acids and derivatives	1.37E-02	5.42E-03	0.758
3.26_164.0712m/z	HMDB00159	L-Phenylalanine	Organic acids and derivatives	1.37E-02	6.87E-03	1.016
1.51_130.0871m/z	HMDB00172	L-Isoleucine	Amino acids and derivatives	1.65E-02	1.03E-02	0.857
0.56_145.0615m/z	HMDB00641	L-Glutamine	Organic acids and derivatives	2.64E-02	1.98E-02	-0.756
3.75_203.0819m/z	HMDB13609	D-Tryptophan	Organoheterocyclic compounds	3.87E-02	3.38E-02	0.677

Note: RT_M/Z = retention time(min)_mass-to-charge ratio; logFC is the log fold change of metabolite values that associates with the ratio of the higher phenotype value over the lower phenotype values. The analysis used an empirical Bayes-paired moderated *t* test adjusted for smoking, drinking and test batch.

L-phenylalanine, L-isoleucine, L-tyrosine, uric acid, NMDA acid, D-(+)-galactose, D-tryptophan remained positively associated with BMI while L-glutamine was negatively associated with BMI in within-twin-pair analysis. Further, the obesity-discordant MZ twin-pair analysis found significantly different levels in most metabolites between co-twins. It has been known for many years that isoleucine (Bogl *et al.*, 2016; Muniandy *et al.*, 2019; Vogelzangs *et al.*, 2020), tyrosine (Ho *et al.*, 2016; Muniandy *et al.*, 2019), phenylalanine (Bogl *et al.*, 2016; Ho *et al.*, 2016; Newgard *et al.*, 2009), tryptophan (Kim *et al.*, 2010) and glutamine (Chashmnam *et al.*, 2020; Oberbach *et al.*, 2011) were biomarkers for obesity, but the studies were mainly conducted in Caucasian populations or Nordic twin

samples. We first confirmed these results in a Chinese population using an MZ co-twin study. Significant within-twin-pair associations between these metabolite levels and BMI point to unique environmental factors rather than the shared factors between co-twins as an underlying contributor.

It has been shown that oxidation during obesity blunts amino acid metabolism in adipose tissue, therefore resulting in the accumulation of the L-isoleucine in the bloodstream (Hanzu *et al.*, 2014; Pietilainen *et al.*, 2008). In Pearson correlation analysis, L-isoleucine showed a significant positive correlation with TC in MZ twin-pair analysis. Both were risk factors for obesity and related type 2 diabetes (Wang *et al.*, 2011).

Table 4. Pearson correlations between 4 amino acids with clinical biochemical measurements in 241 monozygotic twins

RT_M/Z	Metabolite_name	TC		TG		HDL-C		LDL-C	
		MZ indiv.	MZ pair	MZ indiv.	MZ pair	MZ indiv.	MZ pair	MZ indiv.	MZ pair
1.51_130.0871m/z	L-isoleucine	0.13	0.23	0.27	0.18	-0.08	-0.01	0.21	0.17
3.26_164.0712m/z	L-phenylalanine	0.11	0.06	0.18	0.01	0.00	0.09	0.09	0.09
1.23_180.0660m/z	L-tyrosine	0.10	0.08	0.22	0.00	0.00	0.00	0.18	0.09
0.56_145.0615m/z	L-glutamine	-0.24	-0.10	-0.29	-0.05	0.03	-0.16	-0.14	-0.15
0.59_215.0322m/z	C6H12O6*	0.23	0.13	0.10	-0.07	0.20	0.21	0.30	0.22

RT_M/Z	Metabolite_name	Glucose		Insulin		HbA1c		HOMA-IR	
		MZ indiv.	MZ pair	MZ indiv.	MZ pair	MZ indiv.	MZ pair	MZ indiv.	MZ pair
1.51_130.0871m/z	L-isoleucine	0.12	0.02	0.23	0.02	0.18	0.09	0.26	0.02
3.26_164.0712m/z	L-phenylalanine	-0.01	0.01	0.11	0.00	0.15	0.05	0.10	0.01
1.23_180.0660m/z	L-tyrosine	0.04	-0.04	0.28	0.04	0.11	-0.04	0.28	0.02
0.56_145.0615m/z	L-glutamine	-0.28	-0.14	-0.06	0.07	-0.36	-0.17	-0.15	0.01
0.59_215.0322m/z	C6H12O6*	0.79	0.57	-0.02	-0.02	0.63	0.48	0.23	0.18

Note: RT_M/Z:retention time(min)_mass-to-charge ratio; TC = total cholesterol; TG = triglycerides; HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol; HOMA-IR = homeostasis model assessment of insulin resistance. *C6H12O6, galactose/mannose/glucose; MZ indiv., Pearson correlations were calculated treating monozygotic twins as individuals; MZ pair, Pearson correlations were calculated treating monozygotic twins as pairs (115 pairs). The bold type indicates the correlation was significant at $p < .05$.

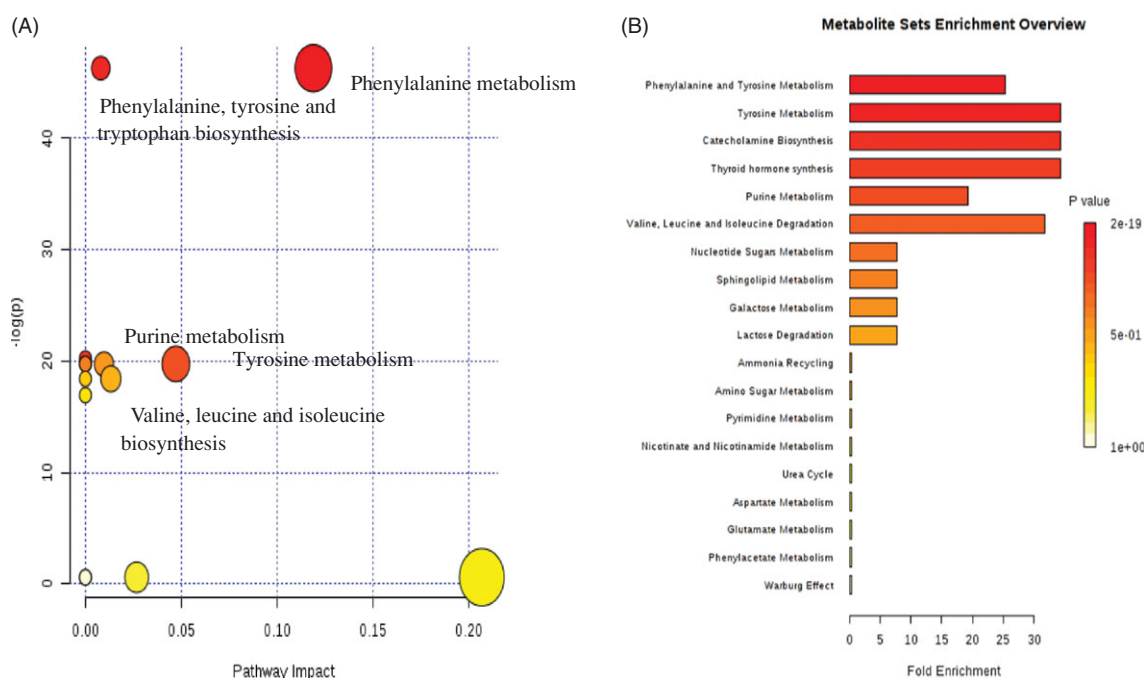


Fig. 2. The results of pathway analysis and enrichment analysis of the metabolomics data. Note: (A) Pathway analysis is based on Kyoto Encyclopedia of Genes and Genomes (KEGG). The color and size of each circle are based on p values (yellow: higher p values and red: lower p values) and pathway impact values (the larger the circle the higher the impact score) calculated from the topological analysis, respectively. Pathways were considered significantly enriched if $p < .05$, impact 0.1 and number of metabolite hits in the pathway > 1 . (B) Enrichment analysis is based on Small Molecule Pathway Database (SMPDB). The color of the bar indicates the p value. The length of the bar indicates the fold enrichment.

Phenylalanine metabolism has the highest impact score in our pathway analysis (Figure 1). High levels of phenylalanine and its downstream product, L-tyrosine, are observed in obese subjects of previous metabolomics studies in twins (Bogl et al., 2016; Muniandy et al., 2019). The liver is an important organ for phenylalanine metabolism, and phenylalanine metabolism has been proposed as a test of liver function (Lara et al., 2000).

Our findings in phenylalanine metabolism might indicate the involvement of liver dysfunction among obese individuals.

Further, enrichment analysis indicated that catecholamine biosynthesis, purine metabolism and sphingolipid metabolism might be involved in obesity development. Catecholamine functions as neurotransmitters and hormones within the body. Alterations in the sympathetic nervous system (SNS) effects on the metabolism

have been implicated in the development and maintenance of obesity and insulin resistance (Da *et al.*, 2020; Li *et al.*, 2020). Experimental studies also found that activation of SNS may promote the development of hypertension in the rat model of obesity (Young *et al.*, 1994). Results of these studies indicate the importance of SNS in regulating metabolic status in obesity. Uric acid in purine metabolism can induce oxidative stress, and activate NADPH oxidase and the renin–angiotensin system, which all play an important role in the pathophysiology of obesity and related metabolic dysregulation (Sautin *et al.*, 2007; Zhang *et al.*, 2015). Sphingolipids are bioactive lipids involving in cellular signaling and regulatory functions (Hannun & Obeid, 2008). Using an experimental model, Choi and Snider (2015) found that sphingolipid metabolism is significantly affected by dietary nutrient oversupply, and dysregulation of sphingolipids contributes to obesity-related cardiovascular disease. The bioactive lipids in sphingolipid metabolism may function as mediators between obesity and increased risk of metabolic dysfunction.

NMDA acid is a new obesity-related metabolite found in our study. NMDA acid is an amino acid derivative acting as a specific agonist at the NMDA receptor. A previous study has found that activation of NMDA receptors in the dorsal vagal complex lowers Glu production and may serve as a therapeutic target for diabetes and obesity (Lam *et al.*, 2010). Experimental studies suggested that NMDA increased the levels of reactive oxygen species (ROS), cytosolic cytochrome c (cyto c) and 17-kDa caspase-3, but depolarized mitochondrial membrane potential, leading to cardiomyocyte apoptosis (Gao *et al.*, 2007; Urushitani *et al.*, 2001). Combined with our results, this may be the pathologic mechanism of obesity-induced cardiovascular disease. However, further studies are warranted to elucidate precise mechanisms and understanding of the role of NMDA in obesity.

Using a twin design combined with an untargeted metabolomic study to better control for confounders, we observed different associations between adiposity and serum metabolite profile. However, several limitations need to be discussed. First, the cross-sectional study design limited the causal interpretation between metabolite profile and obesity traits. Another limitation of our study was the limited number of metabolites identified. Although we used an untargeted approach to assess thousands of metabolite features, only a small fraction can be identified.

In conclusion, obesity is associated with changes in the serum metabolome toward increased risk of metabolic dysfunction. Our findings verified the association of phenylalanine, isoleucine, tyrosine, uric acid and tryptophan with obesity traits in a Chinese twin population, controlling for genetic and shared environmental factors, and additionally identified the important role of NMDA acid. Differing environments within MZ twin pairs might produce epigenetic differences and result in the differing metabolite levels. Further cohort studies with a larger sample size and more diverse biological sample types are needed to replicate our findings.

Acknowledgments. We thank all study participants, research staffs and graduate students who were involved in this study.

Author contributions. CX L was mainly responsible for the design, analyses and drafting of the manuscript. LM L was the principle investigator of this study. WJ G contributed to the conception and design of the study, oversaw the consenting and participated in manuscript polishing. CW H, J L, T H, DJY S and SF W contributed to the conception and design of the study and interpreting the findings. CQ Y participated in data management and cleaning. BQ W contributed to the data analysis. ZC P, LM C, HW and XP W contributed

to the implementation of the study. All authors contributed to the interpretation of results and intellectual content of the manuscript, and have read and approved the final manuscript.

Financial support. This study was supported by the Special Fund for Health Scientific Research in Public Welfare (201502006, 201002007), and the Key Project of Chinese Ministry of Education (310006).

Conflict of interest. None.

Ethical standards. This study was conducted in accordance with the Declaration of Helsinki.

Supplementary material. To view supplementary material for this article, please visit <https://doi.org/10.1017/thg.2021.3>.

References

- Bogl, L. H., Kaye, S. M., Ramo, J. T., Kangas, A. J., Soinen, P., Hakkarainen, A., . . . Pietilainen, K. H. (2016). Abdominal obesity and circulating metabolites: A twin study approach. *Metabolism – Clinical And Experimental*, 65, 111–121.
- Carlin, J. B., Gurrin, L. C., Sterne, J. A., Morley, R., & Dwyer, T. (2005). Regression models for twin studies: A critical review. *International Journal of Epidemiology*, 34, 1089–1099.
- Chashmiani, S., Hashemi, M. N., Nobakht, M. G. B., Safari-Alighiarloo, N., & Khamseh, M. E. (2020). The metabolome profiling of obese and non-obese individuals: Metabolically healthy obese and unhealthy non-obese paradox. *Iranian Journal of Basic Medical Sciences*, 23, 186–194.
- Choi, S., & Snider, A. J. (2015). Sphingolipids in high fat diet and obesity-related diseases. *Mediators of Inflammation*, 2015, 520618.
- Da, S. A., Do, C. J., Li, X., Wang, Z., Mouton, A. J., & Hall, J. E. (2020). Role of hyperinsulinemia and insulin resistance in hypertension: Metabolic syndrome revisited. *Canadian Journal Of Cardiology*, 36, 671–682.
- Gao, X., Xu, X., Pang, J., Zhang, C., Ding, J. M., Peng, X., . . . Cao, J. M. (2007). NMDA receptor activation induces mitochondrial dysfunction, oxidative stress and apoptosis in cultured neonatal rat cardiomyocytes. *Physiological Research*, 56, 559–569.
- GBD 2017 Risk Factor Collaborators. (2018). Global, regional, and national comparative risk assessment of 84 behavioural, environmental and occupational, and metabolic risks or clusters of risks for 195 countries and territories, 1990–2017: A systematic analysis for the Global Burden of Disease Study 2017. *Lancet*, 392, 1923–1994.
- Hannun, Y. A., & Obeid, L. M. (2008). Principles of bioactive lipid signalling: Lessons from sphingolipids. *Nature Reviews Molecular Cell Biology*, 9, 139–150.
- Hanzu, F. A., Vinaixa, M., Papageorgiou, A., Parrizas, M., Correig, X., Delgado, S., . . . Gomis, R. (2014). Obesity rather than regional fat depots marks the metabolomic pattern of adipose tissue: An untargeted metabolomic approach. *Obesity (Silver Spring)*, 22, 698–704.
- Ho, J. E., Larson, M. G., Ghorbani, A., Cheng, S., Chen, M. H., Keyes, M., . . . Wang, T. J. (2016). Metabolomic profiles of body mass index in the Framingham Heart Study reveal distinct cardiometabolic phenotypes. *PLoS One*, 11, e148361.
- Kettunen, J., Tukiainen, T., Sarin, A. P., Ortega-Alonso, A., Tikkanen, E., Lyytikäinen, L. P., . . . Ripatti, S. (2012). Genome-wide association study identifies multiple loci influencing human serum metabolite levels. *Nature Genetics*, 44, 269–276.
- Kim, J. Y., Park, J. Y., Kim, O. Y., Ham, B. M., Kim, H. J., Kwon, D. Y., . . . Lee, J. H. (2010). Metabolic profiling of plasma in overweight/obese and lean men using ultra performance liquid chromatography and Q-TOF mass spectrometry (UPLC-Q-TOF MS). *Journal Of Proteome Research*, 9, 4368–4375.
- Kraus, W. E., Pieper, C. F., Huffman, K. M., Thompson, D. K., Kraus, V. B., Morey, M. C., . . . Newgard, C. B. (2016). Association of plasma small-molecule intermediate metabolites with age and body mass index across six diverse study populations. *Journals of Gerontology, Series A: Biological Sciences*, 71, 1507–1513.
- Lam, C. K., Chari, M., Su, B. B., Cheung, G. W., Kokorovic, A., Yang, C. S., . . . Lam, T. K. (2010). Activation of N-methyl-D-aspartate (NMDA)

- receptors in the dorsal vagal complex lowers glucose production. *Journal Of Biological Chemistry*, 285, 21913–21921.
- Lara, B. S., Razquin, M., Jimenez, I., Vazquez, A., Gisbert, J. P., & Pajares, J. M. (2000). 13C-phenylalanine and 13C-methacetin breath test to evaluate functional capacity of hepatocyte in chronic liver disease. *Digestive and Liver Disease*, 32, 226–232.
- Li, L., Gao, W., Yu, C., Lv, J., Cao, W., Zhan, S., . . . Hu, Y. (2013). The Chinese National Twin Registry: An update. *Twin Research and Human Genetics*, 16, 86–90.
- Li, X. X., Zhang, S. J., Man, K. Y., Chiu, A. P., Lo, L. H., To, J. C., . . . Keng, V. W. (2020). Schwann cell-specific Pten inactivation reveals essential role of the sympathetic nervous system activity in adipose tissue development. *Biochemical and Biophysical Research Communications*, 531, 118–124.
- Liao, C., Gao, W., Cao, W., Lv, J., Yu, C., Wang, S., . . . Li, L. (2015). Associations of body composition measurements with serum lipid, glucose and insulin profile: A Chinese Twin Study. *PLoS One*, 10, e140595.
- Macfarlane, D., Chan, A., & Cerin, E. (2011). Examining the validity and reliability of the Chinese version of the International Physical Activity Questionnaire, long form (IPAQ-LC). *Public Health Nutrition*, 14, 443–450.
- Menke, A., Muntner, P., Wildman, R. P., Reynolds, K., & He, J. (2007). Measures of adiposity and cardiovascular disease risk factors. *Obesity (Silver Spring)*, 15, 785–795.
- Mihalik, S. J., Michaliszyn, S. F., de Las, H. J., Bacha, F., Lee, S., Chace, D. H., . . . Arslanian, S. A. (2012). Metabolomic profiling of fatty acid and amino acid metabolism in youth with obesity and type 2 diabetes: Evidence for enhanced mitochondrial oxidation. *Diabetes Care*, 35, 605–611.
- Moore, S. C., Matthews, C. E., Sampson, J. N., Stolzenberg-Solomon, R. Z., Zheng, W., Cai, Q., . . . Cross, A. J. (2014). Human metabolic correlates of body mass index. *Metabolomics*, 10, 259–269.
- Muniandy, M., Velagapudi, V., Hakkarainen, A., Lundbom, J., Lundbom, N., Rissanen, A., . . . Ollikainen, M. (2019). Plasma metabolites reveal distinct profiles associating with different metabolic risk factors in monozygotic twin pairs. *International Journal of Obesity*, 43, 487–502.
- Nan, C., Guo, B., Warner, C., Fowler, T., Barrett, T., Boomsma, D., . . . Zeegers, M. (2012). Heritability of body mass index in pre-adolescence, young adulthood and late adulthood. *European Journal Of Epidemiology*, 27, 247–253.
- NCD Risk Factor Collaboration. (2017). Worldwide trends in body-mass index, underweight, overweight, and obesity from 1975 to 2016: A pooled analysis of 2416 population-based measurement studies in 128.9 million children, adolescents, and adults. *Lancet*, 390, 2627–2642.
- Newgard, C. B., An, J., Bain, J. R., Muehlbauer, M. J., Stevens, R. D., Lien, L. F., . . . Slentz, C. A. (2009). A branched-chain amino acid-related metabolic signature that differentiates obese and lean humans and contributes to insulin resistance. *Cell Metabolism*, 9, 311–326.
- Oberbach, A., Bluher, M., Wirth, H., Till, H., Kovacs, P., Kullnick, Y., . . . von Bergen, M. (2011). Combined proteomic and metabolomic profiling of serum reveals association of the complement system with obesity and identifies novel markers of body fat mass changes. *Journal of Proteome Research*, 10, 4769–4788.
- Pietiläinen, K. H., Naukkarinen, J., Rissanen, A., Saharinen, J., Ellonen, P., Keranen, H., . . . Peltonen, L. (2008). Global transcript profiles of fat in monozygotic twins discordant for BMI: Pathways behind acquired Obesity. *PLoS Medicine*, 5, e51.
- Pietiläinen, K. H., Sysi-Aho, M., Rissanen, A., Seppänen-Laakso, T., Yki-Järvinen, H., Kaprio, J., & Oresič, M. (2007). Acquired obesity is associated with changes in the serum lipidomic profile independent of genetic effects – A monozygotic twin study. *PLoS One*, 2, e218.
- Rauschert, S., Uhl, O., Koletzko, B., & Hellmuth, C. (2014). Metabolomic biomarkers for obesity in humans: A short review. *Annals of Nutrition and Metabolism*, 64, 314–324.
- Sautin, Y. Y., Nakagawa, T., Zharikov, S., & Johnson, R. J. (2007). Adverse effects of the classic antioxidant uric acid in adipocytes: NADPH oxidase-mediated oxidative/nitrosative stress. *American Journal of Physiology-Cell Physiology*, 293, C584–C596.
- Short, K. R., Chadwick, J. Q., Teague, A. M., Tullier, M. A., Wolbert, L., Coleman, C., & Copeland, K. C. (2019). Effect of obesity and exercise training on plasma amino acids and amino metabolites in American Indian adolescents. *Journal of Clinical Endocrinology and Metabolism*, 104, 3249–3261.
- Urushitani, M., Nakamizo, T., Inoue, R., Sawada, H., Kihara, T., Honda, K., . . . Shimohama, S. (2001). N-methyl-D-aspartate receptor-mediated mitochondrial Ca(2+) overload in acute excitotoxic motor neuron death: A mechanism distinct from chronic neurotoxicity after Ca(2+) influx. *Journal of Neuroscience Research*, 63, 377–387.
- Vogelzangs, N., van der Kallen, C., van Greevenbroek, M., van der Kolk, B. W., Jocken, J., Goossens, G. H., . . . Arts, I. (2020). Metabolic profiling of tissue-specific insulin resistance in human obesity: Results from the Diogenes Study and the Maastricht Study. *International Journal of Obesity*, 44, 1376–1386.
- Wang, T. J., Larson, M. G., Vasan, R. S., Cheng, S., Rhee, E. P., McCabe, E., . . . Gerszten, R. E. (2011). Metabolite profiles and the risk of developing diabetes. *Nature Medicine*, 17, 448–453.
- Young, J. B., Daly, P. A., Uemura, K., & Chaouloff, F. (1994). Effects of chronic lard feeding on sympathetic nervous system activity in the rat. *American Journal of Physiology*, 267, R1320–R1328.
- Zeng, M., Liang, Y., Li, H., Wang, M., Wang, B., Chen, X., . . . Wu, J. (2010). Plasma metabolic fingerprinting of childhood obesity by GC/MS in conjunction with multivariate statistical analysis. *Journal of Pharmaceutical and Biomedical Analysis*, 52, 265–272.
- Zhang, A., Sun, H., & Wang, X. (2012). Serum metabolomics as a novel diagnostic approach for disease: A systematic review. *Analytical And Bioanalytical Chemistry*, 404, 1239–1245.
- Zhang, J. X., Zhang, Y. P., Wu, Q. N., & Chen, B. (2015). Uric acid induces oxidative stress via an activation of the renin-angiotensin system in 3T3-L1 adipocytes. *Endocrine*, 48, 135–142.
- Zhou, B., Li, L., Lyu, J., Yu, C., Wang, S., Pang, Z., . . . Cao, W. (2015). Heritability of body mass index on Chinese adult twins from nine provinces/cities in China. *Zhonghua Liu Xing Bing Xue Za Zhi*, 36, 299–303.